ROLE OF TRACE ELEMENTS ZINC, COPPER AND IRON IN PRE-MATURITY

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CERTIFICATE

This is to certify that the dissertation titled "**ROLE OF TRACE ELEMENTS ZINC, COPPER AND IRON IN PRE-MATURITY"** is a bonafide original work of **Dr. T.V. SRIDEVI** in partial fulfillment of the requirements for **M.D. Branch – VII (Paediatrics)** Examination of the Tamilnadu Dr. M.G.R. Medical University to be held in September 2006. The period of study was from February 2005 to March 2006.

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DECLARATION

I, **Dr. T.V. SRIDEVI** solemnly declare that dissertation titled, **"ROLE OF TRACE ELEMENTS ZINC, COPPER AND IRON IN PRE-MATURITY"** is a bonafide work done by me at Institute of Social Paediatrics, Govt. Stanley Medical College & Hospital during February 2005 to March 2006 under the guidance and supervision of our **Prof. Dr. L. UMADEVI, M.D., D.C.H.,** Professor of Paediatrics.

The dissertation is submitted to Tamilnadu, Dr. M.G.R. Medical University, towards partial fulfilment of requirement for the award of **M.D. Degree (Branch – VII) in Paediatrics.**

Place : Chennai.

Date :

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CONTENTS

INTRODUCTION

Vitamin and Minerals collectively referred to as micro nutrients have important influence on the health of pregnant women and the growing fetus[1]. Pregnancy is associated with increased demand of all nutrients like iron, copper, zinc, vitamin B_{12}, folic acid and ascorbic acid[2] and deficiency of any of these could affect pregnancy, delivery and outcome of pregnancy.

Majority of Indian women practice vegetarianism on dictates or compulsion or religion or poverty and consume cereal based diet rich in phytates, oxalates, phosphates, fiber etc. which affect the absorption of micronutrients like iron[3] and zinc[4].

Many minerals are transferred to the fetus for fetal stores in the latter part of pregnancy, although they may play important developmental role throughout the pregnancy.

Certain essential micronutrients are important constituents of or interact with enzymes. Without proper enzyme functions health and immunity suffers. It has been shown that various minerals such as calcium, phosphorous, magnesium, zinc, copper and iron are metabolically interrelated and there is an alteration in their concentration during pregnancy.

This study was designed to assess the status of the trace elements Zinc, Copper and Iron in maternal and cord blood in preterm and compare it with that of term infants in local population.

REVIEW OF LITERATURE

The importance of mineral balance during pregnancy is still underestimated though diligent research demonstrates that trace elements and minerals are critical for development of the fetus.

Micro nutrients are those with recommended daily allowance below 100 mg / day. More than 99.7% of dry weight is accounted for by 11 elements, termed the major elements. Those which contribute to less than 0.01% of body weight are called trace elements. There are 14 of them namely iron, iodine, zinc, copper, chromium, selenium, manganese, cobalt, molybdenum, nickel, vanadium, silicon, arsenic and fluorine. Optimal biological function depends upon the level of intake or more specifically the tissue concentration of these trace elements. Of these, zinc, copper and iron play a major role in many vital biological functions.

ZINC

The essentiality of zinc for growth and well-being of both plants and animals is well established.

Biochemistry And Physiology

The metabolic functions of zinc are based largerly on its presence as an essential component of many metalloenzymes involved in virtually all aspects of metabolism. Zinc plays a major role in protein synthesis and has an important function in gene expression. Zinc finger proteins that require zinc for their conformation and DNA binding abilities control gene expression.

In addition to its role in catalysis and gene expression, zinc stabilizes the structures of proteins and nucleic acids, preserves the integrity of subcellular organelles, participates in transport processes, wound healing and has important roles in viral and immune phenomena.

Metabolism

Zinc is second to iron as the most abundant trace element in the body, 1.4 to 2.3 g being present in the 70-kg adult. Approximately 20 to 30% of ingested dietary zinc in absorbed. Zinc absorption occurs mostly in the duodenum and proximal jejunum. The absorption process is active, energy dependent, and apparently mediated by specific transport (binding) ligands. Evidence indicates that the zinc absorption mechanism plays a significant role in homeostatic regulation. Zinc absorption is variable and is dependent upon a variety of factors.

High-protein food such meat, fish and diary products are good sources of available zinc. The bio availability of zinc from vegetables and cereal grains is reduced because phytates (inositolphosphates), cellulose, hemicellulose, and other dietary fibers inhibit zinc absorption.

The degree to which phytate inhibits zinc absorption has been defined by the ratio of

phytate to zinc in the diet[5,6]. The World Health Organization[5] recommends an algorithm for estimating zinc bioavailabilty that is based on zinc intake and an availability factor. The availability factor, ie, the percentage of available dietary zinc, is estimated to range from 10% to 50% depending on the phytate-zinc molar ratio in the diet. Zinc availability is projected to be 10% if the phytate-zinc ratio is > 30. 15% if the ratio is 15-30, 30% if the ratio is 5-15, and > 50% if the ratio is < 5. Diet with a phytate – zinc ratio > 15 are high in unrefined, unfermented, and ungerminated cereal grain, especially when fortified with inorganic calcium salts and when intake of animal protein is negligible. Such diets are consumed by many poor women living in developing countries.

The availability of dietary zinc is decreased by high amounts of dietary calcium, phosphorus, iron, and copper. The mechanisms for this iron-zinc interaction probably involved a combination of intraluminal and intracellular effects[7] . Iron and zinc may compete in the absorption process by : 1) displacing one another on the molecule necessary for their uptake from the lumen into the intestinal cell, 2) competing for pathways through the mucosal cell into the blood stream, or 3) interacting with one another and the third substance to form an insoluble complex, impairing the absorption of both minerals.

Western diets generally supply adults with 10 to 15 mg Zn/d. the RDA for zinc for adult males is 15 mg/d.

Zinc is transported in blood plasma mostly by albumin (60-70%) and by- $\alpha 2$ macroglobulin (30-40%), with a small amount associated with transferrin and free amino acids. The major route of zinc excertion is via the feces, others being urine, sweat, semen.

Clinical Significance

Nutritional zinc deficiency in humans is fairly prevalent throughout the world. Primary clinical features include retardation of growth and skeletal maturation, testicular atrophy, and hepatosplenomegaly. Old age, pregnancy, lactation, and alcoholism are also associated with a higher incidence of poor zinc nutrition.

Zinc and Pregnancy

Zinc deficiency has been associated with complications of pregnancy and delivery like spontaneous abortion, pre-term and post-term delivery, prolonged or inefficient first stage of labour, protracted second stage of labour, premature rupture of membranes, PIH as well as with growth retardation and congenital anomalies in the fetus.[1] During pregnancy there is a decline in circulating zinc and also a decline occurs as pregnancy progresses possibly due to decrease in zinc binding and increased transfer of zinc from the mother to the fetus[8,9]. On the basis of total weight of the pregnancy tissues gained during gestation and the zinc concentration in those tissues, it is estimated that the additional need for zinc in a human pregnancy is ≈ 1540 μmol, or ≈100 mg,[10]. This represents ≈ 5-7% of the whole body zinc concentration in a non pregnant women. Most of the zinc gained is deposited in the fetus (57%) and in the uterine muscle (24%).

This additional need for zinc during pregnancy can be met by an increase in zinc intake or by adjustments in zinc homeostasis. Changes in intestinal zinc absorption may be the primary homeostatic adjustment in zinc metabolism to meet the needs for pregnancy. Studies have shown that fractional zinc absorption increases by 30% during gestation and 80% during lactation[11].

Women in developing countries typically receive ≈100mg supplemental iron throughout their pregnancies without any additional zinc. The dietary zinc intake of these women are typically low and the zinc is poorly available. The typical iron-zinc ratio in their diets is estimated to be 24:1, ≈120mg iron: 5mg zinc. This imbalanced intake of iron and zinc may put their zinc status at risk during pregnancy. Interactions between iron and zinc occur during gastrointestinal absorption. The deleterious effect of supplemental iron on zinc absorption and a depression in plasma zinc concentrations were shown in studies of experimental animals and humans.[12,13,14,15].

Zinc and Prematurity

Though zinc deficiency has been implicated in prematurity and post maturity direct evidence is lacking. Some studies[22,25,26] have reported decreased zinc levels in preterm whereas there are also other studies to the contrary[31]. Further few studies[24,27,28] have reported no relation between zinc levels and gestational age. There are also studies which show that zinc supplementation can increase the gestational age [34]. A single micronutrient is unlikely to show an effect in undernourished women with possible multiple micronutrient deficiencies, unless it is the only nutrient limiting fetal growth. The more logical approach is improving overall micronutrient quality of mother's diet.

Laboratory Assessment of Zinc Nutriture

Laboratory tests for assessing zinc nutriture can be classified into two groups: those involving the analysis of zinc in a body tissue or fluid and those testing a zinc dependent function. Useful tests in the first group include determinations of the zinc content of plasma or serum, blood cells, urine and saliva. Functional tests include measurement of activities of zinc-containing enzymes and assessment of taste acuity. The determination of plasma or serum zinc

concentrations by Atomic Absorption Spectrophotometry is the simplest and analytically most reliable test for routine assessment of zinc nutriture. Other less preferred method is photometry.

ANALYSES OF SERUM ZINC BY ATOMIC ABSORBTION SPECTROSCOPY

Zinc level is estimated by the method of *Fernandex et al., (1971)*.

This method describes the determination of zinc in serum. Samples are diluted with deionized water. The analysis is performed against standards prepared with glycerol to approximate the viscosity characteristics of the diluted samples.

Reagents

1. Glycerol diluent : 50 ml of reagent grade glycerol was diluted to 1000 ml with deionized water.

2. Standard solution of Zinc : Zinc metal (AR) 0.5g exactly was dissolved in minimum volume of (1+1) Hcl (AR). Then the solution was diluted to 1 litre with 1% (V/V) Hcl (AR). Concentration – 500µg/ml.

3. Working standard of zinc: 80µl of stock solution was diluted to 100 ml deionized water.

4. Sample preparation : Serum sample were diluted 10 times with deionized water and analyzed.

Analysis

1. Serum samples were brought to room temperature and then mixed by gently inverting the tubes.

2. Deliver 0.5ml of specimen or control into a 16mm plastic test tube, 2.0ml of deionized water was added and mixed immediately.

3. The instrumental and gas flow setting and aspiration rate are established to optimize signal and the background noise was minimized.

4. The instrument was selected with suitable condition. The glycerol diluent was aspirated into the flame and the baseline was read to zero absorbance. The baseline drift was corrected by aspirating the glycerol diluent before and after each aspiration of standard and specimen.

5. The Zinc's working standards was sequentially aspirated from most dilute to most concentrated till the reading is stable. The resulting values are used to establish the working curve.

6. Then the serum sample and control are aspirated into the AAS.

7. The serum Zinc concentration calculated by using the absorbance reading by interpolation from the working curve.

Note : Results for the control should be within 6% of the previously established mean.

SUITABLE INSTRUMENT CONDITIONS

Mineral	Zinc
Hollow Cathode Lamp	Zn
Wave Length	213.9
Flame Type	Air Acetylene
Slit setting	0.2nm
Linear Working Range	0.5 (µg/ml.)
Relative Noise	1.0

The values are expressed as μg/dl

Reference Interval

The accepted reference interval for zinc in plasma is 70 to 150 μg/dl (10.7 – 22.9 μmol/ L). Serum zinc concentrations are generally 5 to 15% higher than plasma because of osmotic fluid shifts from the blood cells when various anticoagulants are used. Plasma zinc concentrations exhibit both circadian and postprandial fluctuations. Concentrations decrease after eating from morning to evening.

COPPER

Biochemistry and Physiology

Copper is an integral component of many metalloenzymes, including ceruloplasmin, cytochrome *c* oxidase, superoxide dismutase, dopamine-β-hydroxylase, ascorbate oxidase, and tyrosinase which are involved in oxidation – reduction reactions.

Copper plays an important role in iron metabolism. Copper deficiency impairs iron absorption, and anemia accompanies severe copper deficiency. Ceruloplasmin the major copper-containing protein in plasma, has a ferroxidase activity that oxidizes ferrous iron to the ferric state prior to its binding by plasma transferrin.

Metabolism

Adult human body contains between 80 and 150mg of copper. Copper absorption is maximal in the duodenum. Other metal ions, particularly zinc and cadmium, compete with copper for sulfhydryl binding sites, thus explaining the antagonism of these metals toward copper absorption.

Between 50 and 80% of ingested copper is absorbed. Factors affecting copper

absorption include gender (women absorbing a greater percentage than men), the amount ingested, chemical form, and certain dietary constituents. The latter include other trace elements, sulfate, various amino acids, fiber, and phytates.

Absorbed copper is rapidly transported as copper-albumin or copper-histidine complexes to the liver where it is stored, mostly as metallothionein-like cuproproteins. Copper is released from the liver, mainly as ceruloplasmin, a multifunctional cuproprotein that accounts for over 95% of the total copper in plasma. Copper may be transported to cells for incorporation into copper-containing enzymes by several identified transport mechanisms.

Copper is excreted primarily in the feces, from unabsorbed dietary copper, and from biliary and gastrointestinal secretions. Other routes of excretion are urine, sweat and menstruation.

The homeostatic regulation of copper metabolism is apparently complex. The liver is the key organ, with copper being stored there and incorporated and released as ceruloplasmin to maintain blood levels. Intestinal absorption mechanisms and regulation of biliary excretion also seem to have major roles in the regulation of copper homeostasis.

Liver, crustaceans, and shellfish contain large amounts of copper, whereas cow's milk and dairy products have low copper contents. The estimated "safe and adequate" range of copper, is 1.5 to 3.0 mg copper/d for adults.

Clinical Significance

A variety of human copper deficiency conditions are recognized. Copper deficiency in infants has been observed in prematurity, malnutrition, malabsorption, chronic diarrhea,

hyperalimentation, and prolonged feeding with low-copper, total-milk diets. Signs of copper deficiency include (1) neutropenia and hypochromic anemia in the early stages, both of which are responsive to oral copper but not iron; (2) osteoporosis and various bone and joint abnormalities that reflect deficient copper-dependent cross-linking of bone collagen and connective tissue; (3) decreased pigmentation of the skin and general pallor, which are attributed to tyrosine-required melanin synthesis; and (4) in the later stages, possible neurological abnormalities (hypotonia, apnea, psychomotor retardation), probably caused by a cytochrome *c* oxidase deficit.

Copper and Pregnancy

Copper deficiency during embryonic and fetal development can result in numerous gross structural and biochemical abnormalities. It is estimated that more than 50% of human conceptions fail to implant, and of those implanting, approximately 30% fail to reach term.[16]. Various documents reveal increasing evidence of a significant number of developmental defects possible due to inadequate nutrition, including trace element copper, during embryonic and fetal development.[17] The fetus is fully dependent on the maternal copper supply. It has been documented that pregnancy is associated with increased copper retention, which may be partly due to decreased biliary copper excretion and partly due to increased hepatic ceruloplasmin synthesis induced by hormonal changes, typical during pregnancy.

Copper deficiency has been reported to result in structural and biochemical abnormalities in the fetus. Studies conducted in the developed nations have documented a significant correlation between low copper in drinking water and the occurrence of neural tube

defects, with the implication being that a primary deficiency of copper could result in birth defects in humans.[18]

Copper and prematurity

The known hypercupremia of gestation[26,30] appears to be more marked in those women who deliver prematurely. Concurrently, maternal ceruloplasmin tends to decrease as gestational age advances. This is consistent with downhill gradient of plasma copper from mother to infant which more marked in 24-28 weeks gestational new borns than in more mature infants[25]. Some studies[24,26] have shown that copper levels are more in preterm. Few other studies[23,25,27] have shown that cord copper levels are less in preterm whereas maternal copper levels bear no relation with gestational age.

Laboratory Assessment of Copper Nutriture

Serum or plasma copper provides a relatively easy routine test for the clinical assessment of copper nutriture; Plasma copper concentration has a diurnal variation with peak values found in the morning. Pregnancy, birth control pills, and infections or inflammatory conditions increase plasma or serum copper, whereas corticosteroids and adrenocorticotropic hormone (ACTH) tend to lower serum copper.

Ceruloplasmin, a copper-containing protein, accounts for over 95% of the copper found in plasma. Thus, it is sensitive to the same factors that affect plasma copper. Enzymatically measured ceruloplasmin has been shown to be a sensitive indicator of copper status in several species of animals.

Measurement of the enzyme activities of the cuproenzymes erythrocyte superoxide

dismutase and cytochrome *c* oxidase in platelets or leukocytes can also provide useful information in assessing copper status. Atomic absorption spectrophotometry after direct dilution is the method of choice for determining serum copper. Other method available is photometric using diethyldithiocarbamate, bis-cyclohexanone, oxaldihydrazone and bathocuprine as chromogens.

ANALYSIS OF SERUM COPPER BY ATOMIC ABSORPTION SPECTROSCOPY

Copper level is estimated by the method of *Parker et al. (1967)*.

This method describes the determination of copper in serum. Sample analysis is performed against standards prepared in glycerol to approximate the viscosity characteristics of the diluted sample.

Reagents

1. Glycerol diluent 10%

2. Standard solution of copper : 1 g of Cu metal was dissolved in min volume of (1+1)HNO_3. It is then diluted to 1 litre with 1% (V/V) HNO_3.

3. Working standard of copper. 400 µl of stock solution diluted to 100 ml with deionized water.

4. Sample preparation : Serum sample were diluted equally with deionized water and analyzed.

Analysis :

Serum specimens diluted with an equal volume of deionized water and are aspirated directly into the atomic absorption spectroscopy flame, and the copper concentrations are calculated against copper standards with a 10 ml/dl glycerol matrix to approximate the viscosity of the diluted specimens.

SUITABLE INSTRUMENT CONDITIONS

Mineral	Copper
Hollow Cathode Lamp	Cu
Wavelength	324.8
Flame Type	Air acetylene
Slit setting	0.7nm
Linear Working Range (µg/ml.)	5.0
Relative Noise	1.0

The values are expressed as µg/dl.

Reference intervals

Reference intervals for serum copper have been compiled and are higher in pregnancy, 118 to 302 µg/dL (18.5 – 47.4 µmol/L); in children 6 to 12 years of age, 80 to 190 µg/dL (12.6 – 29.9 µmol/L); and women, 80 to 155 µg/dL (12.6 – 24.4 µmol/L) than in men, 70 to 140 µg/dL (11.0 – 22.0 µmol/L); and infants, 20 to 70 µg/dL (3.1 – 11.0 µmol/L).

IRON

Biochemistry and Physiology

Iron, a transition and essential metal can take part in redox process by undergoing reversible valency changes. It plays an essential role in oxygen transport by hemoglobin in erythrocytes, oxygen storage by myoglobin in muscles, and election transfer and energy metabolism in mitochondria. It is also a cofactor in various enzymes including catalases, ferroxidases and various protein namely cytochrome and transferrin.

Absorption, Transport and Excretion

The average American diet provides 10 to 15 mg of iron daily, mostly in the form of the

heme proteins, hemoglobin and myoglobin, in meat. In the past, a significant component of iron in the diet came from inorganic iron leached from iron utensils. Normally, approximately 1mg of iron is absorbed each day.

Both ferritin and transferrin are present in the absorptive cells of the intestinal mucosa and are believed together to regulate iron absorption. When body iron are high, the ferritin content of mucosal epithelium is also high and the transferrin content is low. Iron that enters mucosal cells is trapped in ferritin and lost when the mucosal cell is sloughed into the intestinal lumen. This mechanism reduces iron absorption when body stores of iron are already increased. Conversely, with iron deficiency, mucosal cell content of apoferritin is diminished, transferrin (and apotransferrin) content is increased, and iron absorption is accelerated.

The major pathway of iron metabolism is a virtually closed cycle in which iron passes from plasma transferrin to the RBC precursors in the bone marrow, where they remain nearly 4 months before they become metabolically “worn out” and are engulfed by phagocytes; the iron is released from hemoglobin and returns to plasma transferrin, thus completing one cycle and beginning another. From this iron cycle, small quantities of iron are diverted for use in other iron compartments; a slow, continuous exchange also takes place between the storage and transport compartments.

Each day, about 1 to 2 mg of iron absorbed from the intestinal tract enters this cycle to compensate for the 1 to 2 mg of iron lost each day from the body. Most of the iron loss is due to minute quantities of iron present in epithelial cells and RBCs in urine and feces. With each menstrual cycle, young women lose approximately 40 to 80 mL of blood, which is equivalent

to 20 to 40 mg of iron. Similarly, about 600 to 900 mg of iron is lost as a consequence of each pregnancy.

Iron and Pregnancy

Pregnancy may be associated with increased serum iron due to increased progesterone or lower serum iron due to iron deficiency.

Iron supplementation during pregnancy improves the hematological parameters in the mother[19]. Limited data from developed countries suggest a benefit in terms of fetal or neonatal survival[20] but a Cochrane review could not draw any valid conclusion about beneficial effect of routine iron supplementation in pregnancy. Similarly, folate supplementation though decreases the proportion of women having low hemoglobin levels, does not seem to have any effect, beneficial or harmful, on clinical outcomes for mother and baby[21]. Combined supplementation with iron and folate also had no detectable effect on any substantive measures of either maternal or fetal outcome.

Iron and Prematurity

When maternal anaemia is diagnosed before midpregnancy, it has been associated with an increased risk of preterm delivery[37,38]. Maternal anaemia detected during the later stages of pregnancy, especially the third trimester, often reflects the expected expansion of maternal plasma volume. Third trimester anemia usually is not associated with increased risk of preterm delivery. High hemoglobin concentration, elevated hematocrit and increased levels of serum ferritin late in pregnancy, however, all have been associated with increased preterm delivery[40]. This increased risk may reflect in part the failure to expand maternal plasma volume, adequately, thus diminishing appropriate placental perfusion. Thus a U-shaped distribution

exists with higher rates of preterm delivery at both ends of the hemoglobin range[46].

Although controlled trials of iron supplementation during pregnancy have consistently demonstrated positive effects on maternal iron status at delivery, they have not demonstrated reduction in factors that are associated with maternal anemia, i.e. increased risk of preterm delivery and low birth weight[36]. Viteri suggests providing menstruating women at risk with weekly iron folate tablets is likely to improve a woman's iron status before conception and reduce the risk of iron deficiency anaemia in early pregnancy and its attendant complication, i.e. prematurity[39.]

Laboratory Assessment of Iron Nutriture

Serum iron concentration connotes the Fe(III) bound to serum transferrin and does not include the iron contained in serum as free hemoglobin. Serum iron concentration is decreased in many but not all patients with iron deficiency anemia and in chronic inflammatory disorders, in patients who are beginning to respond to specific hematinic therapy for anemias of other causes, acute or recent hemorrhage.

Greater than normal concentrations of serum iron occur in iron loading disorders such as hemochromatosis, in acute iron poisoning in children, and after oral ingestion of iron medication or parenteral iron administration or acute hepatitis.

Serum iron can be estimated accurately by Atomic Absorption Spectrophotometer.

ANALYSIS OF SERUM IRON BY ATOMIC ABSORPTION SPECTROSCOPY

Iron was estimated by the method of Olson et al (1969). This procedure describes the determination of iron in serum. To determine total serum iron, samples, are diluted and heated.

This procedure precipitates the plasma protein and removes approximately 95% of any Hb Iron present.

Reagents

1. 20% TCA
2. 10% TCA
3. Standard Solution of Iron : 1 gm of pure iron powder was weighed accurately and dissolved in 50 ml of (1+1) HNO_3 (GR) and the solution was made upto 1 litre with deionized water. Concentration – 1000µg/ml.

Working standard of iron : 600 µl of stock solution was diluted to 100 ml with deionized water.

Sample preparation:

Serum Iron : In a polyethylene tube, 1.0ml of serum sample was diluted with an equal volume of a 20% (W/V)TCA solution. The tube was capped loosely mixed and heated in a heating block at 90^{o}c for 15 min. It was cooled and centrifuged.

Note : Discard visibly hemolysed samples even though the TCA removes about 95% of Hb iron,

Analysis

The supernatant was analyzed for iron using the conditions listed in the standard condition. It is not necessary to decant the supernatant into another container if care is

exercised to prevent the capillary tubing from contacting the precipitate. Appropriate iron standards are prepared by diluting the iron stock solution described in the standard condition. For iron, with 10% (W/V) TCA.A 10% (W/V) TCA solution should be used for the blank.

Since the samples are diluted 1:2 with TCA, the instrument was calibrated to read 2x the actual concentration of the standards, so as to read the concentration directly.

SUITABLE INSTRUMENT CONDITIONS

Minerals	Iron
Hollow Cathode Lamp	Fe
Wavelength	248.3
Flame Type	Acetylene
Slit setting	0.2 nm
Linear Working Range (µg/ml)	6.0
Relative Noise	1.0

The values are expressed as µg/dl.

PREMATURITY

A preterm neonate is one whose birth occurs through the end of the last day of the thirty-seventh week's (259^{th} day; i.e., 36 6/7 weeks) following onset of the last menstrual period.

Incidence

Approximately 12% of all U.S. births are premature, and about 2% are les than 32 weeks' gestation. In some population segments, demographics play a major role in the incidence of prematurity.

ETIOLOGY

Etiology is unknown in most cases. Premature and / or LBW delivery is associated with the following conditions:

1. Low socioeconomic status (SES), measured by family income, educational level, residency, social class, or occupation.

2. African-American women's rate of very premature (< 32 weeks' gestations) delivery is almost three times that of Caucasian women, and their rate of moderately premature (32-36 weeks) delivery is about one and a half times that of Caucasian women. Disparities persist even when SES is taken into account.

3. Women under age 16 or over 35 are more likely to deliver LBW infants; the association with age is more significant in Caucasians than in African – Americans.

4. Maternal activity requiring long periods of standing or substantial amounts of physical stress may be associated with IUGR and prematurity. This is not significant in mothers from higher-SES groups, possibly because they are les likely to continue working in such jobs once they encounter pregnancy complications.

5. Acute or chronic maternal illness is associated with early delivery, whether spontaneous or, not infrequently, induced.

6. Multiple-gestation births frequently occur prematurely (57% of twins and 93% of triplets in 2000). Birth-weight-specific mortality is not higher in multiples than in singletons; thus their higher rate of neonatal mortality is primarily due to prematurity.

7. Prior poor birth outcome is the single strongest predictor of poor birth outcome. A preterm first birth is the best predictor of a preterm second birth.

8. Obstetric factors such as uterine malformations, uterine trauma, placenta previa, abruptio placenta, hypertensive disorders, preterm cervical shortening or "incompetence," previous cervical surgery, premature rupture of membranes, and amnionitis also contribute to prematurity.

9. Fetal conditions such as IUGR, severe hydrops may require preterm delivery.

10. Inadvertent early delivery because of incorrect estimation of gestational age is now rare.

Problems of Prematurity

1. Respiratory : Premature infants may experience.
    a. Perinatal depression in the delivery room due to poor adaptation of air breathing.
    b. RDS due to surfactant deficiency.
    c. Apnea due to immaturity in mechanisms controlling breathing.
    d. Chronic lung disease, variously described / classified as bronchopulmonary dysplasia, Wilson-Mikity disease, and chronic pulmonary insufficiency of prematurity.
2. Neurologic : Premature infants have a higher risk for neurologic problems including.
    a. Perinatal depression
    b. Intracranial hemorrhage.

c. Periventricular white-matter disease.

3. Cardiovascular: Premature infants may present with cardiovascular problems including.

   a. Hypotension. This may be due to

   1. Hypovolemia,

   2. Cardiac dysfunction, and / or

   3. vasodilation due to sepsis.

   b. Patent ductus arteriosus is common and may lead to congestive heart failure.

4. Hematologic :

   a. Anemia

   b. Hyperbilirubinemia

5. Nutritional : Premature infants require specific attention to the content, amount, and route of feeding.

6. Gastrointestinal : Prematurity is the single greatest risk factor for necrotizing enterocolitis; formula feeding is also a significant risk factor; breast milk is protective.

7. Metabolic problems, especially in glucose and calcium metabolism, are more common in premature infants.

8. Renal : Immature kidney are characterized by low glomerular filtration rate and an inability to handle water, solute and acid loads; Fluid and electrolyte management can be difficult.

9. Temperature regulation : Premature infants are especially susceptible to hypothermia and hyperthermia.

10. Immunologic : Because of deficiencies in both humoral and cellular response, premature infants are at greater risk for infection than are term infants.
11. Ophthalmologic : Retinopathy of prematurity may develop in the immature retina in infants < 32 weeks or < 1500 g birth weight.

Survival of Premature Infants

Mortality was highest (around 50%) in the smallest group of babies (501-750 g) and lowest (< 10%) in the heaviest group (1000 – 1500).

Long – term problems of prematurity

Premature infants are vulnerable to a wide spectrum of morbidity. The risk of morbidity, like that of mortality, declines markedly with increasing gestational age (GA). Although severe impairment occurs in a small population, the prevalence of lesser morbidities is less clearly defined, although large controlled multicenter trials are now providing a more comprehensive picture both of these sequelae and of the effects of intervention.

1. Developmental disability
    a. Major handicaps (cerebral palsy, mental retardation)
    b. Sensory impairments (hearing loss, visual impairment)
    c. Minimal cerebral dysfunction (language disorders, learning disability, hyperactivity, attention deficits, behavior disorders)
2. Retinopathy of prematurity
3. Chronic lung disease

4. Poor growth

5. Increased rates of postneonatal illness and rehospitalization

6. Increased frequency of congenital anomalies

Various studies have been done to assess the levels of zinc, copper and iron in maternal and cord blood of preterm some of which are summarized as follows.

1. ***Jeswani et al***[22] from Ahmedabad in 1991 published a study of serum zinc levels in cord blood of neonates and their mothers in which he reported higher maternal zinc levels and lower cord zinc levels in preterm.

2. ***Algerwie et al***[23] from Bikaner in 1998 conducted a study on serum copper in newborns and their mothers and concluded that maternal copper level is higher than cord copper levels in all babies and that cord copper is more in term as against preterm babies.

3. ***Iqbal et al***[24] from Bangladesh in 2001 assessed serum zinc and copper levels in maternal blood and cord blood of neonates and reported that cord zinc level was higher than maternal levels in all babies and cord copper level was lesser than maternal level. He also noted inverse relation between maternal as well as cord copper levels and gestational age. There was no significant relation between zinc and gestational age.

4. ***Perveen et al***[25] from Manhasset, USA in 2002 conducted a study on effect of gestational age on cord blood plasma, copper, zinc, magnesium and albumin. He

reported that cord zinc level was more in preterm compared to term babies and vice versa for cord copper. He reported that there was no relation of maternal zinc / copper to prematurity.

5. ***Wasowicz et al***[26] from Poland in 1993 performed a study on trace element concentration in maternal and cord blood and determined their relation with birth weight, gestational age and parity. He reported that cord copper was less than maternal copper in all mothers. He also reported cord zinc as well as copper was more in preterm as against term babies.

6. ***Bro et al***[27] of Denmark in 1988 published an article on serum zinc and copper concentrations in maternal and cord blood and its relation to course and outcome of pregnancy. He reported cord copper being less in preterm than term. He also reported that there was no relation between cord zinc, maternal zinc and maternal copper and maturity.

7. ***Srivastava*** **et al**[28] of Faizabad in 2002 reported inverse relation between maternal zinc and iron and maternal age and no relation between trace elements and maturity, but increased maternal zinc, copper and iron in low birth weight babies.

8. ***Chitra upadyaya et al***[29] from Jaipur published a paper 2004 on serum iron, copper and zinc status in maternal and cord blood which showed that cord zinc and iron are more than maternal levels and vice versa for copper.

9. ***Kiilholma et al***[31] in 1984 conducted a study on role of calcium, copper, iron and zinc in preterm delivery and premature rupture of fetal membranes and reported that

maternal zinc is less in preterm and copper less in preterm PROM.

10. **Atinmo et al** [32] in 1980 conducted a study on relationship of zinc and copper concentration in maternal and cord blood and birth weight and reported that cord zinc is more than maternal level whereas it is vice versa in case of copper. He also reported that low maternal as well cord zinc results in low birth weight.

11. ***Abdulla et al***[33] of Sweden conducted a study on the effect of oral zinc supplementation in the year 1998 which showed that zinc supplementation for 4-6 weeks caused increased zinc levels, decreased copper levels where as iron levels are unaltered.

12. ***Garg et al***[34] from Aligarh in 1993 conducted a study on oral zinc supplementation and reported that zinc supplementation increases zinc levels, birth weight and gestational age.

13. ***Doszpod et al*** [35] studied zinc level in maternal and umbilical venous blood in normal, SGA and LGA infants and reported that in SGA babies maternal zinc levels are more than in AGA babies whereas cord zinc follows the opposite trend.

14. ***Klebanoff et al*** [37] performed the case control study and reported that odds ratio for preterm delivery was double in case of second trimester anaemia whereas third trimester anaemia was not a risk factor for preterm delivery.

15. ***Scholl et al*** [38] reported that women with iron deficiency anaemia early in gestation had more than a two fold risk of preterm delivery whereas anaemias due to other causes where not associated with any increased risk of preterm delivery.

AIM AND OBJECTIVES

AIM :

To assess the role of trace elements zinc, copper and iron as an etiology of prematurity.

OBJECTIVES :

- To assess the level of maternal and cord blood zinc, copper and iron and determine its role in etiology of prematurity.
- To determine the relationship between cord and maternal levels of zinc, copper and iron.
- To determine the relationship between cord and maternal zinc, copper and iron and maternal factors influencing prematurity.
- To determine the relationship between cord and maternal zinc, copper and iron and mortality and morbidity of preterm babies.

MATERIALS AND METHODOLOGY

Study Design : Prospective Case Control study

Place : Govt. RSRM Lying-in Hospital,

Institute of Social Paediatrics, Chennai.

Study Period : February 2005 to March 2006

Inclusion Criteria : 1. Newborn with gestational age 28–36 wks.

2. Mother's age between 18 – 35 yrs.

3. Spontaneous premature delivery

Exclusion Criteria : 1. Mothers with BMI < 18

2. Mothers with Chronic liver disease

3. Extreme prematurity GA < 28 wks

4. Instrumental / Operative deliveries

Methodology

The study was performed in Govt. RSRM Lying-in hospital which is the largest maternity hospital located in North Chennai that caters to need of the urban poor of North Chennai.

50 preterm babies with gestational age between 28 to 36 weeks were recruited in the study randomly. 30 term appropriate for gestational age newborns were also included in the study randomly to serve as controls. All the newborns were subjected to the following.

Certain **baseline parameters** of mothers noted like

- Maternal age.
- Socioeconomic scale as per Kuppusamy scale.
- Order of pregnancy
- FST intake – whether taken or not, if taken whether adequate or not
- H/o intake of other drugs
- Medical / Obstetric illness
- Any obvious cause of prematurity if present, was identified.

Collection of blood

After obtaining informed consent from the mother 10 ml of cord blood was collected from placental side of umbilical cord after cord clamping to coincide precisely with the newborn venous blood level and 10 ml of maternal blood collected from antecubital vein at the same time.

Post natal examination follow up of mother

After delivery, the height and weight of the mothers were recorded from which BMI was calculated. Mothers were also followed up for any postnatal complications.

Postnatal examination and follow up of new born.

All newborns were subjected to detailed examination including the following.

- Assessment of gestational age as per New Ballard Scoring.
- Anthropometric assessment including weight, length and head circumference.
- Sex of the baby.
- Presence of any congenital malformation.

Babies followed up till the time of discharge / death for development of any complication related to prematurity.

Cleaning of Glassware

As per recommended guidelines all syringes, blood collecting and centrifuge tubes, aspirator and serum storage vials were first cleaned with detergent solution and later rinsed thrice with a mixture of (1+1) nitric acid and deionized water. The residual layer of acid was allowed to remain in content with distilled water. Then they were rinsed with several portions of distilled water and finally they were rinsed three times with deionized water. All the containers used during the analysis were cleaned as above.

Deionized water was prepared by passing distilled water through charcoal filter and single bed deioniser was used throughout the analysis.

Handling of blood specimens

- Maternal hemoglobin was determined by Sahlis' method.
- Both maternal and cord blood were stored in refrigerator and centrifuged within 24 hours of collection at 3000 rpm for 10-15 mins. at clinical pathology laboratory of Institute of Social Paediatrics, Govt. Stanley Hospital. Serum was separated and stored in sample vial in freezer compartment of refrigerator till analysis was done.

Trace element level determination

Trace element levels zinc, copper and iron was determined by Atomic absorption spectrophotometer at department of animal nutrition, Tamilnadu University of Veterinary and Animal Sciences, Vepery, Chennai using the method described previously.

Statistical Analysis

Difference in levels of quantitative data like maternal age, BMI, hemoglobin, weight, maternal and cord zinc, copper and iron between cases and controls were analyzed using student ‘t’ test.

Qualitative data like socioeconomic scale, order of pregnancy, booked / un booked; FST intake and sex of the baby analyzed using Pearson chi square test.

Outcome differences on selected factors like morbidity, cause of prematurity and gestational age were analysed using one way analysis of variance of F test.

Correlation between outcome variable and maternal and baby factors were analysed using Pearson correlation coefficient and Biserial correlation coefficient.

OBSERVATION AND RESULTS

Table –I a – SES in Pre-term Vs Term

SES Class	Pre-term		Term		Total
	No.	%	No.	%	
3	7	14	1	3.33	8
4	36	72	27	90	63
5	7	14	2	6.67	9
Total	50	100	30	100	80

X^2 = 3.80 ***P = 0.15*** ***Not Significant***

Table – I b – Order of Pregnancy in Pre-term Vs Term

Gravida	Preterm		Term		Total
	No.	%	No.	%	
1	25	50	12	40	37
2	14	28	11	36.67	25
3	9	18	3	10	12
4	1	2	3	10	4
5	1	2	1	3.33	2
Total	50	100	30	100	80

X^2 = 4.9 ***P = 0.38*** ***Not Significant***

Table – I c – Booking in Pre-term Vs Term

Booking	Pre-term		Term		Total
	No.	%	No.	%	
Booked	37	74	24	80	61
Un Booked	13	26	6	20	19
Total	50	100	30	100	80

$X^2 = 0.37$ ***P = 0.54*** ***Not Significant***

Table – I d - FST Intake in Pre-term Vs Term

FST	Preterm		Term		Total
	No.	%	No.	%	
No	5	10	2	6.67	7
Inadequate	18	36	18	60	36
Adequate	27	54	10	33.33	37
Total	50	100	30	100	80

$X^2 = 4.3$ **P = 0.11** ***Not Significant***

Table I e - Sex of the Baby in Pre-term Vs Term

Sex	Pre-term		Term		Total
	No.	%	No.	%	
Male	27	54	13	43.33	40
Female	23	46	17	56.67	40
Total	50	100	30	100	80

$X^2 = 0.85$ ***P = 0.35*** ***Not Significant***

Tables 1a to e show that both the groups – cases comprising pre term and control comprising term babies were comparable in terms of qualitative parameters like socio economic scale, order of pregnancy, whether booked / un booked, FST intake and sex of the baby.

Table – 1 f – Quantitative Data in Pre-term Vs Term

Data	Group	N	Mean	Std. Deviation	Student t-test	Signifi-cance
Maternal age	Pre-term	50	23.64	3.498	t = 0.98	NS
	Term	30	22.90	2.808	p = 0.30	
BMI	Pre-term	50	23.02	3.61	t = 1.26	NS
	Term	30	24.04	3.32	p = 0.21	
Hb%	Pre-term	50	9.72	0.93	t = 0.48	NS
	Term	30	9.81	0.71	p = 0.63	

Table – I f, shows that both the groups were also comparable in terms of quantitative data like maternal age, Body mass index and hemoglobin.

Table – II a -Maternal trace elements in Pre-term Vs Term

Element	Group	N	Mean	Std.	Student	Significance

			µg/dL	Deviation	t-Test	
Zinc	Preterm	50	0.62	0.37	t = 3.56	***Significant***
	Term	30	0.37	0.09	p = 0.01	
Copper	Preterm	50	2.85	1.02	t = 1.32	NS
	Term	30	2.58	0.58	p = 0.19	
Iron	Preterm	50	0.73	0.34	t = 1.96	***Significant***
	Term	30	0.87	0.30	p = 0.05	

Table II a, shows that mean maternal zinc, copper and iron levels in preterm and term babies.

It can be observed that mean maternal zinc is more in preterm which is statistically significant where as mean maternal iron is statistically less in preterm.

There is no statistically significant difference in mean maternal copper levels in preterm and term.

Table – II b – Cord Trace elements in Preterm Vs Term

Element	Group	N	Mean µg/dL	Std. Deviation	Student t-Test	Signifi-cance
Zinc	Preterm	50	1.25	0.53	t = 6.41	***Significant***
	Term	30	0.60	0.20	p = 0.001	
Copper	Preterm	50	0.63	0.29	t = 0.74	NS
	Term	30	0.59	0.22	p = 0.47	
Iron	Preterm	50	1.22	0.41	t = 3.42	***Significant***
	Term	30	1.85	1.18	p = 0.001	

Table – II b, shows that mean cord blood levels of zinc, copper and iron in preterm and term babies.

Mean cord zinc level is more in preterm which is statistically significant.

Mean cord iron level is less in preterm which too is statistically significant.

Mean cord copper level does not vary significantly in preterm in comparison to term babies.

Table – III a- Relation Between Maternal And Cord Zinc in Pre-term and Term

Group	Element	N	Mean µg/dL	Std. Deviation	Student T-test	Signifi-cance
Preterm	Maternal Zinc	50	0.62	0.37	t = 6.06 p = 0.001	***Significant***
	Cord zinc	50	1.25	0.53		
Term	Maternal zinc	30	0.37	0.09	t = 5.81 p = 0.001	***Significant***
	Cord zinc	30	0.60	0.20		

Table – III a shows that in both preterm and term groups cord zinc levels are significantly higher compared to maternal levels.

Table – III b – Relation Between Maternal and Cord Copper in Pre Term and Term

Group	Element	N	Mean µg/dL	Std. Deviation	Student t-test	Signifi-cance
Preterm	Maternal Copper	50	2.85	1.02	t = 14.79 p = 0.001	***Significant***
	Cord Copper	50	0.63	0.29		
Term	Maternal Copper	30	2.58	0.58	t = 17.55 p = 0.001	***Significant***
	Cord Copper	30	0.59	0.22		

Table – III b, shows that cord copper levels are significantly lower than maternal levels in both preterm as well as term babies.

Table – III c – Relation Between Maternal and Cord Iron in Pre-term and Term

Group	Element	N	Mean µg/dL	Std. Deviation	Student T-test	Significant
Pre-term	Maternal Iron	50	0.73	0.34	t = 6.52 p = 0.001	***Significant***
	Cord Iron	50	1.22	0.41		
Term	Maternal Iron	30	0.81	0.30	t = 4.41 p = 0.001	***Significant***
	Cord Iron	30	1.85	1.18		

Table – III c, indicates that in both preterm as well as term babies cord iron levels are significantly higher when compared to maternal levels.

Table – IV a – Maternal Trace Elements Vs Maternal Factors

Factor	Zinc			Copper			Iron		
	r - value	p - value	sig.	r - value	p - value	sig.	r - value	p - value	sig.
MATERNAL AGE	0.18	0.21	NS	-0.02	0.89	NS	-0.29	0.04	***Sig.***
SES	0.06	0.67	NS	-0.16	9.26	NS	0.07	0.64	NS
Gravida	0.09	0.52	NS	0.15	0.30	NS	-0.13	0.37	NS
BMI	-0.06	0.66	NS	0.01	0.92	NS	-0.12	0.40	NS
Booked	-0.25	0.08	NS	-0.14	0.32	NS	-0.04	0.79	NS
Hb	- 0.02	0.90	NS	-0.24	0.10	NS	0.31	0.03	***Sig.***
FST	-0.33	0.02	***Sig.***	-0.02	0.92	NS	0.01	0.94	NS
Disease	-0.10	0.50	NS	0.45	0.001	***Sig.***	-0.07	0.65	NS

Table – IV-a shows that maternal zinc has significant inverse relation with FST intake meaning that more FST intake is related to decreased maternal zinc levels.

The correlation between maternal zinc and selected factors is represented in correlation matrix.

Maternal iron has significant inverse relation with maternal age, which means maternal iron levels become less as maternal age advances. Maternal iron levels has a significant direct relation with hemoglobin levels as expected. The relation of maternal iron levels with maternal age, hemoglobin and FST intake is brought out in the correlation matrix.

Table IV b – Maternal Trace Elements Vs Baby Factors

Factor	Zinc			Copper			Iron		
	r - value	p - value	sig.	r - value	p - value	sig.	r - value	p - value	sig.
GA	-0.21	0.15	NS	-0.27	0.06	NS	-0.17	0.23	NS

Sex	0.16	0.27	NS	0.04	0.78	NS	-0.07	0.64	NS
Weight	-0.21	0.15	NS	-0.43	0.002	***Sig.***	-0.10	0.50	NS
Morbidity	-0.07	0.65	NS	0.35	0.01	***Sig.***	-0.08	0.57	NS
Mortality	-0.05	0.73	NS	-0.29	0.04	***Sig.***	0.19	0.18	NS

Table IV b shows that maternal copper has significant direct relation with morbidity and inverse relation with mortality and birth weight meaning increased maternal copper levels are associated with neonatal complications, neonatal death and low birth weight. This correlation is very well brought out in the correlation matrix.

Table – IV c – Cord Trace Elements Vs Maternal Factors

Factors	Zinc			Copper			Iron		
	r - value	p - value	Sig.	r - value	p - value	Sig.	r - value	p - value	Sig.
Maternal age	0.03	0.81	NS	0.13	0.37	NS	-0.18	0.20	NS
SES	0.10	0.49	NS	-0.07	0.64	NS	0.03	0.82	NS
Gravida	-0.07	0.65	NS	0.03	0.81	NS	-0.22	0.12	NS
BMI	0.27	0.06	NS	-0.01	0.94	NS	-0.27	0.05	NS
Booked	-0.04	0.80	NS	-0.14	0.32	NS	0.03	0.84	NS
Hb	0.07	0.65	NS	-0.24	0.09	NS	-0.01	0.96	NS
FST	-0.33	0.05	***Sig.***	-0.22	0.13	NS	0.15	0.30	NS
Disease	0.27	0.06	NS	0.14	0.34	NS	0.09	0.54	NS

Among cord trace elements, zinc is the only element that has a significant correlation with maternal / baby factors.

Cord zinc has a significant inverse relation with FST intake.

This relation is depicted in the correlation matrix.

Table – IV d – Cord Elements Vs Baby Factors

Factors	Zinc			Copper			Iron		
	r - value	p - value	Sig.	r - value	p - value	Sig.	r - value	p - value	Sig.
GA	-0.26	0.07	NS	-0.06	0.70	NS	0.01	0.93	NS
Sex	0.12	0.40	NS	0.06	0.69	NS	-0.18	0.21	NS
Weight	-0.34	0.02	**Sig.**	-0.05	0.73	NS	0.17	0.24	NS
Morbidity	0.16	0.28	NS	-0.03	0.85	NS	-0.19	0.19	NS
Mortality	-0.28	0.06	NS	-0.02	0.91	NS	0.24	0.09	NS

Table IV d shows that cord zinc has significant inverse relation with birth weight meaning that cord zinc level is more in low birth weight babies. This is also depicted in the correlation matrix.

Table – V – Identifiable Cause Of Pre Maturity

	No. of Cases	%
None	25	50.0
Previous Prematurity	6	12.0
Anemia	6	12.0
PIH	6	12.0
APH	1	2.0
PROM	2	4.0
Other Obst. Illness	3	6.0
Multiple Pregnancy	3	6.0

Table – V shows that of 50 preterm babies no cause could be identified in 25 whereas in the remaining 25 cause could be identified, common causes were previous prematurity, maternal anemia and PIH – less common ones being multiple pregnancy, PROM, antipartum hemorrhage and other obstetric illness.

Same data is represented as a pie-chart.

No significant relation was found between the trace element levels and cause of prematurity.

Table – VI-a, Frequency of various gestational age

Gestational Age	Frequency	Percentage
28 – 30 Weeks	6	12.0
30 – 32 Weeks	5	10.0
32 – 34 Weeks	17	34.0
34 – 36 Weeks	22	44.0
Total	50	100.0

Table – VI – a shows that of 50 cases 22 were of gestational age 34-36 weeks, 17 were of 32-34 weeks, 5 of 30 – 32 weeks and 6 of 28 – 30 weeks.

Same data is represented as pie-chart.

Table – VI-b Relation between maternal trace elements and various gestational age

Element	GA (Weeks)	N	Mean	Std. Deviation	F-test	Significance
Zinc	28 – 30	6	0.5683	0.15587	F=9.05 P=0.001	***Sig.***
	30-32	5	1.1420	1.02236		
	32-34	17	0.6047	0.18344		
	34-36	22	0.5332	0.15885		
	> 37	30	0.3733	0.09234		
Copper	28 – 30	6	3.6667	1.98209	F=3.32 P=0.015	***Sig.***
	30-32	5	2.3560	0.41313		
	32-34	17	3.0941	0.60354		
	34-36	22	2.5650	0.90102		
	> 37	30	2.5870	0.57950		
Iron	28 – 30	6	0.9200	0.54100	F=1.49 P=0.213	NS
	30-32	5	0.7600	0.41755		
	32-34	17	0.6894	0.33469		
	34-36	22	0.7082	0.26043		
	> 37	30	0.8737	0.29805		

Table –VI-b shows relation between maternal zinc, copper and iron and preterm babies with various gestational age groups.

Significant relation was observed with maternal zinc and copper.

Maternal trace elements in various gestational age is represented graphically.

Table – VI-c Relation between Cord trace elements and various gestational age

Element	GA (Weeks)	N	Mean	Std. Deviation	F-test	Significance
Zinc	28 – 30	6	1.4617	0.54190	F=12.60 P=0.001	***Sig.***
	30-32	5	1.3260	0.46742		
	32-34	17	1.3888	0.61043		
	34-36	22	1.0768	0.44446		
	> 37	30	0.6077	0.19646		
Copper	28 – 30	6	0.7250	0.34841	F=1.20 P=0.316	NS
	30-32	5	0.7360	0.18366		
	32-34	17	0.5329	0.20211		
	34-36	22	0.6700	0.34337		
	> 37	30	0.5910	0.22427		
Iron	28 – 30	6	1.1620	0.25170	F=2.90 P=0.027	***Sig.***
	30-32	5	1.1680	0.38280		
	32-34	17	1.3150	0.56840		
	34-36	22	1.1900	0.33470		
	> 37	30	1.8550	1.1850		

Table – VI- c, shows relation between cord zinc, copper and iron with various gestational ages.

Significant relation was observed with cord zinc and iron.

Cord trace elements in various gestational is represented graphically.

DISCUSSION

About 10 to 12 percent of Indian babies are born preterm (less than 37 completed weeks) as compared to 5 to 7 percent incidence in the west[16]. These infants are anatomically and functionally immature and therefore their neonatal mortality is high.

The mechanisms initiating normal labour are not clearly understood and much less is known about the triggers that initiate labour before term.

This study was aimed to assess the trace element levels zinc, copper and iron in maternal and cord blood of preterm and to determine if any association existed between abnormal trace element levels and prematurity.

Study group babies were preterm babies of gestational age 28-36 weeks from lower socioeconomic strata (Modified Kuppusamy's scale III, IV, and V). Term appropriate for gestational age babies who served as control also belonged to similar socio economic strata. Both groups were comparable in terms of other baseline data like maternal age, BMI, order of pregnancy, whether booked / unbooked, maternal hemoglobin and sex of the baby.

In this study majority of babies were of gestational age 34-36 weeks (44%) followed by those in 32-34 weeks(34%), 28-30 weeks (12%) and 30-32 weeks (10%) in order of frequency.

Causes of prematurity could be identified in half of the cases whereas none could be identified in others. No significant relation was found between trace element levels and cause of prematurity.

In this study, mean maternal zinc was noted to be significantly more in preterm as

against term babies. This is in concurrence with studies of Jeswani et al[22] 1991 of Ahmedabad and Wasowicz et al[26] 1993 of Poland, but against that of Kiilholma et al[33]. But Iqbal et al[24] in 2001, Bangladesh, Perveen et al[25] of USA and Bro et al[27] of Denmark reported no significant relation between maternal zinc and gestational age.

Mean maternal copper levels had no relation with gestational age in this study. The same finding was reported by Perveen et al[25] of USA and Bro et al[27] of Denmark.

In this study, mean maternal iron level was significantly less in preterm as against term deliveries. There were no studies favour of this finding. But Srivastava et al[28] in 2002 reported that maternal iron levels had no relation with gestational age. But many studies[37,38,40] reported low haemoglobin and ferritin levels being associated with prematurity.

On analyzing cord trace element levels mean cord zinc level was significantly more in preterm compared to term babies. Studies of Perveen et al[25] of USA and Wasowicz et al[26] of Poland were in concurrence with this finding whereas those of Jeswani et al[22] of Ahmedabad and Bro et al[27] of Denmark were against it. However Iqbal et al[24] from Bangladesh reported no significant relation between cord zinc levels and gestational age. Drecosti et al[32] in 1982 indicated that the uphill gradient characteristic of zinc transport during gestational in more marked earlier in last trimester than late. This explained why cord levels are more in preterm when compared to term babies.

In this study cord copper did not have any relation with gestational age. But some studies by Iqbal et al[24] and Wasowicz et al[26] report a direct relation where as some others by Algerwie et al[23], Bro et al[27], Perveen et al[25] report an inverse relation between cord copper and

gestational age.

Cord iron level was found to be significantly less in preterm in this study. There are no previous studies on relation between cord iron level and gestational age.

Thus decreased maternal and cord iron noted in preterm may suggest iron deficiency as a possible cause of preterm delivery.

Increased maternal and cord zinc noted in preterm infants show that zinc deficiency cannot be implicated in preterm delivery in our study population and increased levels may be due to pattern of transfer across placenta and zinc binding capacity[1, 5].

In this study, cord zinc levels were significantly higher compared to maternal levels in preterm as well as term babies. This is in concurrence with studies done by Iqbal et al[24] from Bangladesh and Chitra Upadyaya et al[29] from Jaipur.

Cord copper levels were significantly lower compared to maternal levels in preterm as well as term babies. This is in concurrence with studies done by Algerwie et al[23] of Bikaner, Iqbal et al[24] of Bangladesh, Wasowicz et al[26] of Poland and Chitra Upadyaya et al[29] of Jaipur.

Cord iron levels were significantly higher compared to maternal levels in all babies. This is in concurrence with study done by Chitra Upadyaya et al[29] from Jaipur.

During late pregnancy when fetal demand for zinc and iron is high and dietary supply is low, the plasma turns over more frequently and transfers more zinc and iron out of the circulation, probably to the fetus to support growth and development. Zinc is actively transferred from mother to fetus across the placenta and there is also decreased zinc binding

capacity of maternal blood during pregnancy which facilitates efficient transfer of zinc from mother to fetus resulting in an increased level of zinc in cord blood[8,9].

Low cord copper levels noted in our study may be caused by parallel increase in major copper binding protein ceruloplasmin which might be increased as a result of elevated levels of maternal estrogen. The parsimonious delivery of copper to fetus is consistent with its inability to synthesise greater amounts of ceruloplasmin or alternatively a physiological consequence of greater zinc influx into fetal compartment which inhibits copper translocation by an increase of placental metallothionen[25].

This study also showed that maternal as well as cord zinc has a inverse relation with FST intake meaning supplementation of pregnant women with iron alone without adding zinc may lead to zinc deficiency in them. This is supported by various studies [12,13,14,15].

It was also noted that maternal iron level is inversely related to maternal age. Similar association was reported by Srivastava et al[28] thus increased maternal age predisposes to prematurity by decreasing maternal iron levels in addition to being a risk factor by itself.

Another curious finding noted in the study was increased maternal copper was associated with neonatal complications and death and low birth weight. The exact reason for this is unclear. Atinmo et al[32] in his study reported increased maternal copper level in low birth weight babies. Further studies are needed to throw more light on this.

SUMMARY AND CONCLUSION

- Decreased maternal and cord iron levels is noted in preterm as against term implicating iron deficiency as a possible cause of prematurity and reinforcing the need for iron supplementation in pregnant women and preterm babies.

- Increased maternal and cord zinc levels in preterm eliminates zinc deficiency as a cause of prematurity and implies that routine zinc supplementation in mothers may not have role in prevention of prematurity.

- The fact that maternal and cord copper levels do not vary significantly in preterm compared to term eliminates copper as a probable cause of prematurity.

- Cord levels of zinc and iron are more than corresponding maternal level in all babies.

- Cord level of copper is less than corresponding maternal level in all babies.

- The finding that increased FST intake is associated with zinc deficiency implies that mere iron supplementation during pregnancy without adding zinc may tilt the precarious zinc balance during pregnancy and lead to overt zinc deficiency.

- Increasing maternal age was found to be associated with decreasing maternal iron levels and this could have a role in causation of prematurity.

- Increased maternal copper level is associated with low birth weight, neonatal morbidity and mortality which needs to be confirmed by further large scale studies.

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PROFORMA

Type of Sample : Test / Control Sl.No.

Patient Name : IP No. :

Age : DOD :

Socioeconomic Scale: F - E O I Total Score -

M - E O Class –

Obstetric History : G P L A

Maternal Ht : Wt : BMI :

Maternal Hb :

Supplementation : None / Iron alone / Iron and Zinc

No. of Tablets

Any other medication :

Medical Illness :

Obstetric Illness :

Other Illness :

Post natal outcome :

Gestation : Single / Multiple

Identifiable cause of Prematurity :

Gestational Age :

Category : SGA / AGA / LGA

Anthropometry Length :

Weight :

Head Circumference :

Any Congenital Malformation :

Outcome of Baby : Alive / Dead

Disease, if any

KEY TO MASTER CHART

Booking	:	1	-	Booked
		0	-	Unbooked
FST	:	0	-	None
		1	-	Inadequate Intake
		2	-	Adequate Intake
Cause of Prematurity	:	0	-	None
		1	-	Previous Prematurity
		2a	-	Anemia
		2b	-	PIH
		2c	-	APH
		2d	-	Anemia + PIH
		2e	-	Chorioamnionitis
		2f	-	Others
		2g	-	Anemia+ Multiple Pregnancy
		3	-	Multiple Pregnancy
Gestational Age :		1	-	28 – 30 Weeks
		2	-	30 – 32 Weeks
		3	-	32 –2 34 Weeks
		4	-	34 – 36 Weeks
Sex	:	1	-	Male
		2	-	Female
Morbidity	:	1	-	(RDS) Respiratory Distress Syndrome
		2	-	Apnea
		3	-	Perinotal Depression
		4	-	Others
Mortality	:	0	-	Dead
		1	-	Alive

ABBREVIATIONS

AAS	:	Atomic Absorption Spectrophotometer
AGA	:	Appropriate for Gestational Age
APH	:	Ante Patron Hemorrhage
BMI	:	Body Mass Index
FST	:	Ferrous Sulphate Tablet
GA	:	Gestational Age
Hb	:	Hemoglobin
HCl	:	Hydrochloric acid
HNO_3	:	Nitric acid
LGA	:	Large for Gestational Age
nm	:	Nanometer
PIH	:	Pregnancy Induced Hypertension
PROM	:	Premature Rupture of Membrane
RPM	:	Revolutions per minute
SES	:	Socio Economic Scale
SGA	:	Small for Gestational Age
TCA	:	Trichloroacetic acid
V/V	:	Volume / Volume
W/V	:	Weight / Volume
µgm	:	Microgram